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# ASSESSMENT OF ZINC CONTENT IN DIFFERENT GENOTYPES OF SUPER ELITE RICE OF VARYING LEVELS OF POLISHING GROWN UNDER CONTRASTING WATER REGIME

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***ABSTRACT***

***Zinc deficiency is one of the major concerns leading to causing major diseases including death. Across the developing countries, rice is a staple food and a vital source of nutrients however deficient in Zinc. Biofortification is the effective approach to combat hidden hunger. When rice (Oryza Sativa L.) grains are polished, they lose important nutrients. Deficit irrigation, a method of artificially generating drought in crops, has been shown to increase micronutrient content. Droughts that occur naturally may have a positive impact on grain nutritional value. The current study used 12 super elite rice genotypes cultivated at GKVK in RCBD during kharif 2015 and summer 2016 under contrasting water regimes (aerobic and wetland environments) to show strong signs of biofortified rice. Zinc concentration in Brown Rice was determined using an X-Ray Fluorescence Spectroscopy technique with varying levels of polishing. High Zinc concentration in brown rice in kharif, Azucena; 40.23 ppm, and AM 1; 33.17 ppm; in summer, and varying levels of polishing – 5 percent polished, AM 72; 34.93 ppm in kharif and AM 65; 27.80 ppm in summer, – 10 percent polished, AM 72; 31.53 ppm in kharif and AM 65; 27.80 ppm in summer***

***KEYWORDS: Biofortification, Micronutrient, Oryza sativa L. & Zinc Deficiency***

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Original Article

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is the greatest, the most nutritive and unquestionably the most widespread staple in the world. In the area of rice cultivation, India ranks first with 43.97 million ha and second in production with 104.32 million tons[1].

Rice production largely depends on the irrigated lowland rice system but water scarcity and other water related problems caused due to pollution, climate change, global warming, increased cost of cultivation and poor availability of labour have threatened the very conventional technology production using regular wetland puddled transplanted system of rice. Aerobic rice cultivation is a new technology which reduces water use and there is no standing water like conventional way of cultivation, and it is a direct seeding methodology which in turn reduces the cost of cultivation with lesser labour requirement. It also minimizes the greenhouse gas emission rates from rice field which make an ecofriendly approach[10].

Rice consumption per capita is extremely high, ranging from 62 to 190 kg per year in some regions where rice is the main meal. As a result, it is regarded as one of the most important crop plants on the planet. Rice contributes roughly 29.3 percent of dietary calories and 29.1 percent of dietary protein in some developing nations, which is

significantly higher as a dietary contribution9. Essential amino acids, vitamins, and trace elements, particularly ionic elements like Zinc (Zn), Iron (Fe), Iodine (I), and Selenium (Se), as well as Vitamin A, C, D, E, B Vitamin, and folic acid, as well as essential amino acids, trace elements, the uncommon S-rich amino acids, lysine, and methionine25, should all be included in the diet. However, nutritional deficiency-related illnesses are common among the population, particularly in developing nations where cereal-based diets are the norm3. Micronutrient malnutrition, often known as hidden hunger21, is now affecting one-half of the world's population, primarily women and young children, especially babies. In developing countries, deficits in iron, zinc, and vitamin A have been observed in the human population. In countries23, zinc deficiency is the fifth leading cause of disorders such as anorexia, dwarfism, a weakened immune system, skin lesions, hypogonadism, and diarrhoea, as well as fatalities. Zinc bioavailability is frequently reduced due to cereal protein's high phytate concentration. Alcoholism, malabsorption, sickle cell anaemia, and chronic renal illness are all known to increase the risk of zinc deficiency. Acrodermatitis enterpathica, for example, is a severe deficit that can be fatal22. The only long-term answer to malnutrition in underdeveloped nations is a significant improvement in food quality. Biofortification is the process of selecting plants with high levels of bioavailable micronutrients in their seeds, hence addressing the root cause of micronutrient shortages. 7. Because they have particular nutrient enrichment features within their genomes that can be used to improve micronutrient levels without affecting crop yield, these staple crops can be enriched with micronutrients utilising plant breeding procedures.[11].

Biofortification, coupled with plant breeding, is one of the innovative and unique ways for improving the nutritional condition and health of underprivileged populations in both rural and urban parts of the developing world21. The biofortification has three primary essentially related advantages: I cost-effectiveness It has the potential to reach relatively remote poor rural populations who rely on rice as a staple diet, (ii) be sustainable, and (iii) have an impact in relatively remote rural areas where food staples do not enter the marketing system or processing facilities are small, numerous, and widely dispersed[6].

CGIAR introduced biofortification program through its HarvestPlus initiative with which international agriculture and research centers are been able to develop an improved breed of staple crops are predominantly rich in vitamins and minerals[23].

Rice grain comprises of (i) hull which includes lemma and palea, and (ii) the rice caryopsis, which is known as brown rice. The hulled rice consists of 6-7 per cent bran, 90 per cent endosperm and 2-3 per cent embryo and further removal of bran layer yields in white rice. White rice means 8-10 per cent removal of bran. In general, the more rice brown is removed from the grain during polishing, the more mineral nutrients are lost14. Rice is commonly used as milled or white rice, which is made by dehulling and milling rough rice kernels to remove the hull and bran covering. Rice bran contains protein and fats, whereas the endosperm contains starch. The removal of bran by milling reduces fat and protein levels while increasing starch levels in the residual kernel20.

It has been discovered that polishing rice is one of the key causes of rice deficit in vital mineral elements. Around 11,400 samples of brown and milled rice were analysed for iron and zinc levels, with brown rice containing only 20-25 parts per million (ppm) zinc and milled rice containing 16-17 ppm zinc17. Due to a non-uniform distribution of nutrients in the kernel15, the degree of milling and polishing has a considerable impact on the nutritional properties of white rice, particularly minerals. The polished rice fraction consists primarily of the endosperm of the rice grain, whereas the bran includes the

majority of the embryo and aleurone layer. Bran > hull > whole grain > brown rice > polished rice16 were the relative Zn contents in the different grain segments.

Grain zinc level in rice germplasm accessions ranged from 0.4 to 104 mg/kg, and Bekele et al., (2013) reported 16.1 to 88.6 mg/kg for the RIL population in rice.[2,5].

In view of the overhead aspects, the contemporaneous study is conducted for determination of Zinc content in brown and varied level of polished rice in super elite genotypes along with their mean performance with phenotypic traits under two contrasting water regimes.

## 2. MATERIAL AND METHODS

- The experiment took place at the field of rice research laboratory, department of Plant Biotechnology, UAS, GKVK, Bengaluru, which is located at 12o 58' North, 77o 35' East, and 930 metres above mean sea level (MSL).
- *Plant material*

Seven super elite rice lines possessing high along with the two checks and three parents varied in its Zinc content were selected prior studies 4 undertaken using more than 1200 rice accessions. The list of selected super elite rice genotypes is given below in Table 1.

**Table 1: List of Selected Elite Lines with Checks Differing for Grain Zinc Content**

| SL. No | Variety Name | Pedigree |
|---|---|---|
| 1 | AM 1 | Azucena X Moromutant |
| 2 | AM 27 | Azucena X Moromutant |
| 3 | AM 65 | Azucena X Moromutant |
| 4 | AM 72 | Azucena X Moromutant |
| 5 | AM 94B | Azucena X Moromutant |
| 6 | AM 143B | Azucena X Moromutant |
| 7 | ARB 6 | Budda X IR 64 |
| 8 | IR 64 | Parent (IRRI-developed indica variety) |
| 9 | Chittimuthyalu | Check (Rice Variety from Andra Pradesh) |
| 10 | Azucena | Parent (Japonica Variety) |
| 11 | Moroberekan | Parent (Japonica Variety) |
| 12 | Budda | Parent (Local Germ Plasm from Karnataka) |

### A. Field experimental Conditions

The selected super elite 12 genotypes are grown in kharif 2015 and summer 2016 seasons under aerobic and wetland conditions. The experiment was laid out in Random completely blocked design (RCBD) with three replications in two seasons, kharif 2015 and summer 2016. Under aerobic condition, all the twelve genotypes were raised in direct seeding method in the main field irrigation was done once in three days for all the plants. However, under traditional wetland condition, a nursery was raised by direct seed bed method and healthy plants were transplanted using line sowing method to the puddled condition after 21 days of sowing. To ensure the right nutrition management is met to maintain the health of the plants, fertilizers were provided on time and necessary steps were taken to control pest and disease infestation.

### B. Estimation of Zinc Content

The seeds harvested from the main field were de-husked (hulled) using Palm Husker to obtain brown rice (Unpolished Rice).

Brown Rice were subjected to polishing using nonferrous miller (Mini Lab Rice Polisher Model K-710, Krishi International). The moisture of the grain were checked using moisture meter and ensured the moisture is between 10-12%. Estimation of Zinc content is based on the X-ray fluorescence (XRF) method[27]. Zinc content was estimated in five-gram (5 g) Brown rice and varied level (5% & 10%) of polished rice using X-ray florescence (XRF) (OXFORD Instruments X-Supreme 8000 at MSSRF, Chennai, Tamil Nadu. The average of three replications is considered for result[5].

### C. Statistical Analysis

For each of the 12 super elite rice genotypes, mean values of five plants utilised for recording observations were computed for several phenotypic variables under each of the water regimes in both seasons. Statistical analysis was performed on the tabulated data. Panse and Sukhatme19 used mean data in their study of variance to split the variance attributable to the different sources. The significance of the results was determined by comparing them to the table values provided by Yates27. Standard error of means (SEM) and critical difference (CD) were used to compare the individual line means using the relevant formulas. The phenotypic and genotypic coefficients of variation were calculated according to Burton and Dewane8. In a broad sense Hanson12 developed a method for calculating heritability, and Johnson explained a formula for calculating genetic progress[13].

## 3. RESULTS AND DISCUSSION

### D. Analysis of Variance

For each trait, the Analysis of Variance is investigated. Table 2 A, B, C, D shows the mean sum of squares for Zinc content in brown rice, 5% polished rice, and 10% polished rice of 12 genotypes due to various sources of variation.

**Table 2 A: Analysis of Variance for Brown Zinc and Varied Level of Polishing under Aerobic Condition in Kharif 2015**

| Source of Variation | Degree of Freedom | Mean Sum of Squares | | |
|---|---|---|---|---|
| | | B Zn | 5% Pol Zn | 10% Pol Zn |
| Genotype | 11 | 40.07** | 24.90** | 34.50* |
| Error | 22 | 10.76 | 7.74 | 11.80 |
| CD @ 5% | | 5.55 | 4.71 | 5.82 |
| CD @ 1% | | 7.55 | 6.40 | 7.91 |
| CV | | 9.30 | 8.95 | 12.36 |

**Table 2 B: Analysis of Variance for Brown Zinc and Varied Level of Polishing under Wetland Condition in Kharif 2015**

| Source of Variation | Degree of freedom | Mean Sum of Squares | | |
|---|---|---|---|---|
| | | B Zn | 5% Pol Zn | 10% Pol Zn |
| Genotype | 11 | 36.95* | 52.91** | 37.02** |
| Error | 22 | 15.54 | 5.47 | 1.63 |
| CD @ 5% | | 6.68 | 3.96 | 2.16 |
| CD @ 1% | | 9.07 | 5.39 | 2.94 |
| CV | | 14.02 | 9.27 | 5.82 |

**Table 2 C: Analysis of Variance for Brown Zinc and varied level of polishing under Aerobic Condition in Summer 2016**

| Source of Variation | Degree of freedom | Mean Sum of Squares | | |
|---|---|---|---|---|
| | | B Zn | 5% Pol Zn | 10% Pol Zn |
| Genotype | 11 | 78.66** | 91.36** | 78.85** |
| Error | 22 | 13.88 | 11.68 | 10.01 |
| CD @ 5% | | 6.31 | 5.79 | 5.36 |
| CD @ 1% | | 8.57 | 7.86 | 7.28 |
| CV | | 13.72 | 16.67 | 17.57 |

**Table 2 D: Analysis of Variance for Brown Zinc and varied Level of Polishing under Wetland Condition in Summer 2016**

| Source of Variation | Degree of freedom | Mean Sum of Squares | | |
|---|---|---|---|---|
| | | B Zn | 5% Pol Zn | 10% Pol Zn |
| Genotype | 11 | 54.44** | 65.31** | 60.80** |
| Error | 22 | 12.86 | 11.99 | 6.15 |
| CD @ 5% | | 6.07 | 5.86 | 4.20 |
| CD @ 1% | | 8.25 | 7.97 | 5.71 |
| CV | | 15.47 | 19.21 | 17.20 |

** Significant at 1%, * Significant at 5%

B Zn – Zinc Content in Brown Rice, 5% Pol Zn – Zinc Content in 5% Polished Rice, 10% Pol Zn – Zinc Content in 10% Polished Rice

All of the features showed highly significant changes between genotypes, showing a large range of variability among the genotypes studied.

**E. Genetic Parameters**

Tables 3A,B,C,D shows the genetic parameters for zinc content in Brown rice and polished rice, including minimum, maximum, mean value, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in the broad sense (h2), and genetic advance as percent mean (GAM) for all genotypes.

According to Robinson et al., PCV and GCV were classified (1949). 0-10% was regarded low, 10-20% was deemed moderate, and 20% and above was considered excessive.

The percentage of heritability was classified as follows: 0-30% was regarded low, 30-60% was deemed moderate. As high as 60% and higher

The GA was classed as a percent mean13, as shown below.

5% to 10% Low percentage (10-20%) 20% and more is considered high.

Estimates of genetic factors for Brown Zinc concentration and various levels of polishing for chosen genotypes under Aerobic Conditions in Kharif 2015 are shown in Table 3 A.

| | *Kharif* 2015_Aerobic Condition | | | | | | |
|---|---|---|---|---|---|---|---|
| Trait | Mean±SE | Range | | GCV (%) | PCV (%) | $h_2$ Broad sense | GAM (%) |
| | | Min. | Max. | | | | |
| B Zn | 35.26±1.9 | 28.80 | 40.23 | 8.86 | 12.85 | 47.58 | 12.60 |
| 5% Pol Zn | 31.07±1.61 | 25.70 | 34.93 | 7.70 | 11.81 | 42.50 | 10.34 |
| 10% Pol Zn | 27.8±1.99 | 19.77 | 31.53 | 9.89 | 15.83 | 39.06 | 12.74 |

**Table 3 B: Estimates of Genetic Parameters for Brown Zinc Content and varied Level of Polishing for Selected Genotypes under Wetland Condition in *Kharif* 2015**

| | *Kharif* 2015_Wet Land Condition | | | | | | |
|---|---|---|---|---|---|---|---|
| Trait | Mean ± SE | Range | | GCV (%) | PCV (%) | $h_2$ Broad sense | GAM (%) |
| | | Min. | Max. | | | | |
| B Zn | 28.13 ± 2.28 | 22.97 | 33.70 | 9.50 | 16.93 | 31.47 | 10.97 |
| 5% Pol Zn | 25.23 ± 1.36 | 16.97 | 30.53 | 15.76 | 18.29 | 74.28 | 27.98 |
| 10% Pol Zn | 21.93 ± 0.74 | 16.30 | 27.90 | 15.66 | 16.71 | 87.86 | 30.24 |

**Table 3 C: Estimates of Genetic Parameters for Brown Zinc Content and varied level of Polishing for selected Genotypes under Aerobic Condition in Summer 2016**

| | Summer 2016_Aerobic Condition | | | | | | |
|---|---|---|---|---|---|---|---|
| Trait | Mean ± SE | Range | | GCV (%) | PCV (%) | $h_2$ Broad sense | GAM (%) |
| | | Min. | Max. | | | | |
| B Zn | 27.15 ± 2.16 | 14.93 | 33.17 | 17.12 | 21.94 | 60.88 | 27.51 |
| 5% Pol Zn | 20.5 ± 1.98 | 13.20 | 27.80 | 25.14 | 30.16 | 69.46 | 43.16 |
| 10% Pol Zn | 18.01 ± 1.83 | 11.73 | 26.90 | 26.60 | 31.88 | 69.62 | 45.72 |

**Table 3 D: Estimates of Genetic Parameters for Brown Zinc content and Varied Level of Polishing for Selected Genotypes under Wetland Condition in Summer 2016**

| | Summer 2016_Wet Land Condition | | | | | | |
|---|---|---|---|---|---|---|---|
| Trait | Mean ± SE | Range | | GCV (%) | PCV (%) | $h_2$ Broad sense | GAM (%) |
| | | Min. | Max. | | | | |
| B Zn | 23.19 ± 2.08 | 12.83 | 31.03 | 16.05 | 22.29 | 51.87 | 23.82 |
| 5% Pol Zn | 18.03 ± 2 | 10.83 | 25.60 | 23.38 | 30.26 | 59.72 | 37.23 |
| 10% Pol Zn | 14.43 ± 1.44 | 9.07 | 24.27 | 29.58 | 34.21 | 74.76 | 52.68 |

GCV: Genotypic coefficient of variation

PCV: Phenotypic coefficient of variation

h2: Heritability percentage

GAM: Genetic advance

Low GCV % is observed in all the traits for selected genotypes under aerobic condition for kharif 2015 season wherein moderate to high GCV % along with PCV % is observed for all the traits under aerobic aswell as wetland condition for summer 2016.

*C. Zinc Content in Brown Rice, Polished Rice (5%), and Polished Rice (10%)*

*Figure. 1, Figure. 2, Figure. 3, and Figure. 4 show zinc concentration in brown rice, 5% polished rice, and 10% polished rice for various genotypes in kharif 2015 and summer 2016 under differing water regimes.*

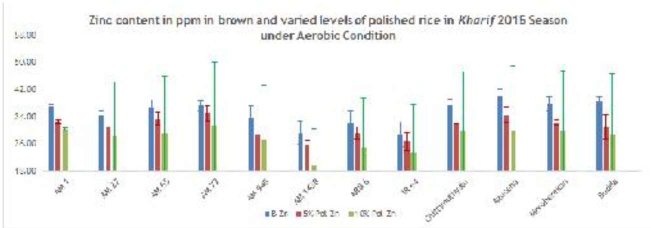


**Figure 1: Zinc Content in ppm in brown and varied levels of Polished rice in Kharif 2015 Season under Aerobic Condition.**

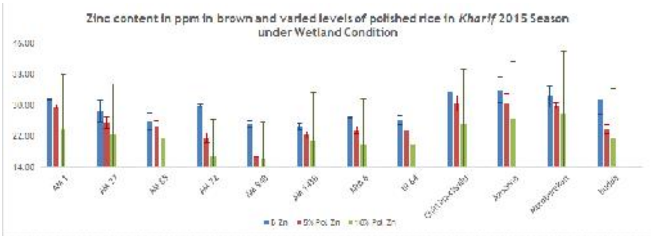


**Figure 2: Zinc Content in ppm in Brown and varied Levels of Polished rice in Kharif 2015 Season under Wetland Condition.**

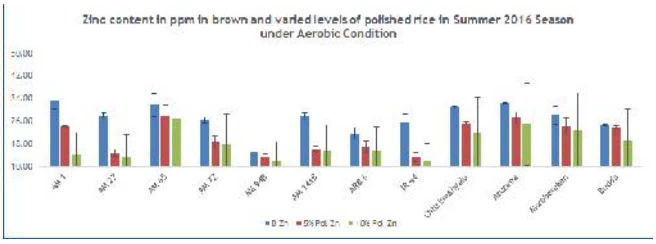


**Figure 3: Zinc Content in ppm in brown and Varied Levels of Polished Rice in Summer 2016 Season under Aerobic Condition**

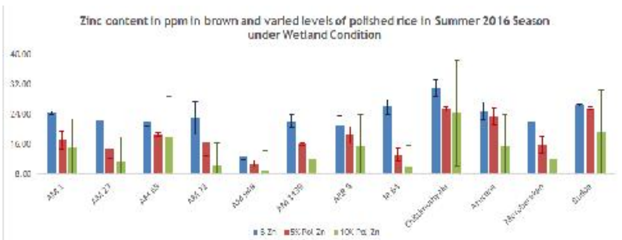


**Figure 4: Zinc Content in ppm in Brown and Varied Levels of Polished Rice in Summer 2016 Season under Wetland Condition.**

When compared to wetland, brown rice had a higher zinc concentration (Azucena; 40.2 ppm in kharif and AM 1; 33.17 ppm in summer) and a different level of polishing (AM-72; 31.53 to 34.93 ppm in kharif and AM 65; 26.80 to 27.80 ppm in summer).

## 4. CONCLUSIONS

In comparison to wetland, high zinc content in brown rice (Azucena; 40.2 ppm in kharif, and AM 1; 33.17 ppm in summer) and a varying level of polishing (AM-72; 31.53 to 34.93 ppm in kharif, and AM 65; 26.80 to 27.80 ppm in summer) were seen in aerobic conditions in both seasons. Highest grain yield is reported under Aerobic condition in Azucena (22.77 gm) followed by ARB 6 (16.88 gm) in Kharif, and AM 27 (23.67 gm) followed by AM 72 (19.84 gm in Summer. In this present study, High Zinc and Iron is reported in Brown Rice under Aerobic condition and it is observed that the per cent loss in Iron content was more due to polishing of rice as compared to Zinc. The experiment gave a strong indication of bio-fortified rice with high yield under Aerobic condition as compared to wetland conditions in both Kharif 2015 and summer 2016 seasons. Further, it is also observed that few Genotypes (AM 72 and AM 65) is strongly aromatic.

It is evidently seen that per cent of loss in zinc content due to varied level of polishing was less in the super elite genotypes.

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